Aceh Journal of Animal Science, 4(1): 18-26
DOI: 10.13170/ajas.4.1.13558
Printed ISSN 2502-9568 Electronic ISSN 2622-8734

RESEARCH PAPER

Irradiation of intraerythrocytic *Plasmodium berghei* with a fractionated dose of gamma rays does not effectively reduce the infectivity in mice *Mus musculus*

Mukh Syaifudin*, Siti Nurhayati, Darlina Darlina, Yanti Lusiyanti, Teja Kisnanto
Nuclear Medicine Technique and Radiation Biology Division, Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency (BATAN), Jl. Lebakbulus Raya No. 49 Po Box 7043 JKSKL Jakarta, Indonesia. *Corresponding author's email: mukh_syaifudin@batan.go.id

Received: 27 April 2019
Accepted: 15 June 2019

ABSTRACT

Malaria infection kills more than one million human every year, mainly under-5-year-old children, including in South East Asian nations. Gamma radiation given at a single dose is commonly used to create the attenuated Plasmodium parasites to get vaccine materials. However, there is no study on the infectivity of parasites after fractionated γ-radiation. This study aimed to assess the infectivity of parasites after irradiated with fractionated γ-rays in mice. A number of *Plasmodium berghei* that was irradiated in two fractions of 100 and 50 Gy, 100 and 75 Gy; and 100 and 100 Gy within 5 minutes of interval time was injected intraperitoneally into 12 mice. Mice injected with unirradiated parasites (0 Gy) served as a control group. The parasitemia level of intraerythrocytic parasites in each group was observed at days post injection up to 20 days by making Giemsa stained thin blood smears and observed under the microscope. Results showed that fractionation radiation did not effectively attenuate the parasites where they still grew in blood of mice, except for 100+75 Gy. There are no significant differences among the treatment groups ($p>0.05$). This is different from irradiation at the single dose that resulted in almost completely attenuated parasites mainly the dose of 150 Gy. This implicating that irradiation of gamma rays at a single dose is a better way to mitigate parasites than fractionation dose as the infectivity of irradiated parasites were lower compared to that of fractionated dosage.

Keywords: Malaria vaccine, Gamma radiation, Fractionation, Parasitemia

INTRODUCTION

Malaria is a protozoan infection with protean manifestations in the human, cause more than one million mortalities per year mainly children under five years old, especially in Africa (Ramani S *et al.*, 2016). Approximately 50% of the world's population is very susceptible to malaria; for example, in 2017, there were an estimated 219 million cases of malaria in 87 countries (WHO, 2018). Even though decades of intense efforts to control human malaria, the disease is still one of the significant health problems in Africa and parts of South East Asia counties. The successful in eradicating *P. falciparum* malaria worldwide will almost certainly require the development of an efficacious vaccine that includes a blood-stage (intraerythrocytic) component (Feachem *et al.*, 2010). Gamma radiation is an effective method used to generate the whole parasite-based malaria vaccines (Seo, 2015; Oakley *et al.*, 2013; Mahmoudi and Keshavarz, 2018).

Ionizing radiation is harmful to the growth and survival of parasites. By using murine models, it is known that asexual blood-stage parasites exposed to high doses of gamma rays failed to induce blood-stage parasite infection (Gerald, 2011), and injection of radiation-attenuated blood stage parasites adequately protected against parasitemia (Gerald, 2011; Bijker *et al.*, 2015; Ouattara and Laurens, 2015) and cerebral malaria (Gerald, 2011). More than two decades ago, researchers had

found that volunteers built up high levels of protection to malaria after being bitten by mosquitoes containing radiation-weakened parasites in its salivary glands. Since then, they had been striving to utilize vaccines made from whole living parasites. To ensure that the parasites are sufficiently weakened for the vaccine, they must be exposed to a radiation dose of at least 150 Gray of gamma rays (dose rate of 300-350 Gy h^{-1}), but not more (Anonymous, 2007; Syaifudin *et al*., 2011).

The dose-response relationship of biological parameters following exposure to ionizing radiation is affected by some factors. Of these, the most important are the target cells under study, dose range, dose rate and dose spacing (fractionation), as well as the temporal relationship of the change and the strain of organism utilized (Hall and Brenner, 1991). In vaccine development, irradiation is usually conducted at once (single dose) that may kill parasites and so live-attenuated parasites are not obtained for vaccine materials. One alternative to overcome this case is dividing the doses of irradiation into two or more parts (fractionation) at a lower dose than killing dose.

Exposure to ionizing radiation has a severe effect on the viability and further proliferation of living cells (Cabiscol *et al*., 2000; Mettler and Voelz, 2002). All tissues exhibit varying degrees of susceptibility to ionizing radiation. Each has a threshold that, if crossed, will produce irreversible damage. Fractionation was discovered early in the 20th century that a more substantial total radiation dose can be delivered if divided over several treatments/fractions, than when a single dose is given. This is so because the organism's tissues get a chance to recover between treatments (Fulda *et al*., 2010; Han and Yu, 2009). Protracted and fractionated doses cause less effect allowing time for intracellular repair and tissue recovery (Moding *et al*., 2013). In this case, the live-attenuated parasites are intended to be used as vaccine materials.

For a long time, the laboratory mouse has been widely used for malaria vaccine research where the pathogen under study is intractable to routine laboratory manipulation. However, the experimental study of the human malaria parasite *P. falciparum* is particularly problematic due to the fact that its complete life cycle cannot be maintained *in vitro*. Thus rodent models of malaria such as *P. berghei* have been used successfully to complement research on *P. falciparum* (Carlton *et al*., 2002).

A serial research on malaria vaccine had been done in the Center where the main focus, according to tasks, is the determination of dose and dose rate of gamma rays that is most effective in attenuating *P. berghei*, either for pre-erythrocytic- or erythrocytic-stage malaria parasites (Syaifudin *et al*., 2011; Syaifudin *et al*., 2013). These are considered by several aspects such as hematopoietic, histopathologic, immunologic and genetics. In hematopoietic point of view, an effective single dose of gamma rays had been obtained (150 Gy) at a dose rate of 380 Gyh^{-1}. However, this high dose of radiation delivered as a single treatment to the mice is risky as the low number of attenuated parasites. To get broader insight, this paper describes the results on the infectivity of irradiated *P. berghei* in mice, the most appropriate model, after fractionated exposure of gamma rays. Hence, the objective of the present study was to assess the infectivity of parasites after irradiated with fractionated γ-rays in mice

MATERIALS AND METHODS

Parasite and animal model

Mouse blood infected with *Plasmodium berghei* (Antwerpen-Kasapa, ANKA strain) at the density of $\pm 10^6$ parasites ml^{-1} were obtained from Eijkman Institute for Molecular Biology, Indonesian Ministry of Research and Technology. Male Swiss-Webster mice (6–8 weeks old at the beginning of experimental study) were purchased from Tropical Medicine Laboratory, National

Institute of Health Research and Development, Ministry of Health and were housed in animal room of the Center for Technology of Radiation Safety and Metrology, BATAN and handled according to experimental animal standard guidelines. All treatments in this experiment were reviewed and approved by the Animal Care and Use National Commission, Institute of Health Research and Development, the Ministry of Health.

Experimental design

The completely randomized design was used in this study. Twelve mice were divided into four groups consists of three mice in each. One group was as control, and others group were irradiated with gamma rays with dose variation as described in irradiation subsection.

Infection of mice

Mice were intraperitoneally (IP) injected with parasitized mouse blood containing about 10^6 *P. berghei* ml^{-1}. Parasitaemia was monitored started in the third day after infection on the blood smears after being stained with Giemsa solution using light microscopy and was repeated every 2-3 days up to day 20.

Irradiation of intraerythrocytic-stage parasites

Mouse blood infected bloods with 10-25% parasitemia were put into a 2 mL sterilized vial and irradiated *in vitro* in a gamma irradiator (Cobalt-60 source) of the Center for Application of Isotope and Radiation, BATAN to fractionated doses of 0 (non-irradiated), 100+50, 100+75 and 100+100 Gy at interval time of 5 minutes and at dose rate of 380.0 Gy h^{-1}. Then the irradiated parasites were injected intraperitoneally to mice. Mice injected with non-irradiated blood were used as control.

Parasitemia observation.

Parasitemias in infected mouse blood from each group were observed started on day two post-injection and repeated every 2-3 days up to 20 days by Giemsa staining. Thin blood smears were prepared by tail-end bleeding, room temperature dried, and methanol fixed before staining step that was done with a 10% Giemsa solution for 10 minutes. Slides were observed at a 1000× magnification (on a drop of oil-immersion) using a light microscope by reading around 20 fields for every slide. The total number of red blood cells counted was about 4000 cells for every treatment.

Statistical analysis.

Statistical analysis on the significant difference between doses of radiation was done using software SPSS version 16.0 for Windows. The distribution of data is determined with the Kolmogorov-Smirnov test. In case of the normal distribution is obtained then parametric or One-way ANOVA test is applied, whereas in case of the not normal distribution is obtained then the Mann-Whitney U test was used.

RESULTS

Results showed that parasitemia was detected in the blood of the mice starting on day 7 after injection of irradiated *P. berghei* except for 100+75 Gy that took longer time (day 9) and reached values above 10% by day 11 (Figure 1a). The increment of parasitemia was seen for all days of

observation in all treatment groups, mainly 100+50 Gy. All mice developed parasitemia even though the parasites had been irradiated with gamma rays and it takes a longer time compared to non-irradiated parasites, indicating an effect of irradiation on the biological activity of parasites. From day 9 to 13, parasitemia rose slightly mainly at the dose of 100+75 Gy and was not significantly different from other doses (100+50 and 100+100 Gy) ($p>0.05$). Among the three treatments conducted in this experiment, the fractionated dose of 100+75 Gy was most effective in inhibiting the growth of parasites in the blood of mice. The survival of mice of 100+50 Gy group was shorter than other groups, indicating the failed of fractionated dose in inhibiting the parasite growth.

In this experiment, an extremely low parasitemia was found in 150 and 175 Gy doses. The parasitaemia for dose of 150 Gy was not significantly different ($p<0.05$) with 175 Gy, but significantly different ($p=0.029$) with both control (0 Gy) and 200 Gy. Inoculation of non-irradiated (0 Gy) *P. berghei* resulted in a very high infection and all mice were dead on day 17. The mice showed, on days 5–10 of infection, clinical signs of malaria such as loss of sense and paralysis of the lower half of the body. Parasitaemia in this control mice was started to be detected three days after infection and divided rapidly for all infected mice up to day 20 of infection. All mice inoculated with γ-ray irradiated parasites at 150 and 175 Gy as a single dose were survived up to the end of the experiment. The use of γ-ray irradiated parasites at 150 Gy as one dose revealed a significant decrease immunity level compared with their analogous at 100+50 Gy till day 17 or more after injection. Exposure to high doses of γ-radiation may cause the attenuation and even mortality of intraerythrocytic *P. berghei*. At three days after treatment with 150 and 175 Gy of γ-radiation, approximately 99.5% reduction in parasitemia was observed (Figure 1b). For both radiation doses the parasites still alive or survived and had a normal growth indicating they were able to repair the cell damage and then repopulate. These were highly different from that of mice injected with 200 Gy irradiated parasites.

If current results are compared with that of a single dose (once) of irradiation at 150 Gy as an optimal dose to attenuate parasites, these three fractionated doses treatments (100+50, 100+75 and 100+100 Gy) did not effectively inhibit the growth of parasites. Parasites growth (parasitemia) for doses of 100+50 and 100+75 Gy were significantly different ($p<0.05$) with their corresponding single doses (150 and 175 Gy), but no significant difference was found between 100+100 and 200 Gy ($p=0.591$). The fractionated dose spares parasites to growth as normal parasites (control, 0 Gy) as shown in Figure 1a. However, for single dose of 150 dan 175 Gy irradiation treatment there was the extremely low number of parasites detected in the blood of mouse during observation days as evidenced by impaired parasite development, even though they have grown up less than 10% at the end of the experiment. The exception is for 200 Gy irradiation, of which the parasites were still grown up to more than 50% at day 40 post-infection (Figure 1b).

Irradiation was proven to effectively inhibit the growth of parasites so that the mice had a much longer lifetime compared to the non-irradiation group (42 days versus 28 days). Considering the forms of parasites during its intraerythrocytic stage, at early days (day 7–9), young trophozoites or ring forms were mostly observed; on the subsequent days, a clear predominance of a parasite stage was not observed. It is essential to be noted that most of the infected mice survived until day 20 except all mice in 100+50 Gy that dead at day 16 with clinical signs of cerebral malaria. From day 13 and afterward, young erythrocyte forms appeared in the peripheral blood of mice and parasitized red blood cells were getting larger (Figure 2). It is well known that the stages of the parasite that most sensitive to irradiation are the ring forms and the early trophozoites; whereas late trophozoites are relatively not sensitive. The study also showed that the highest resistance parasites are those of irradiated at a time of transition from the late trophozoite and schizont to young ring forms.

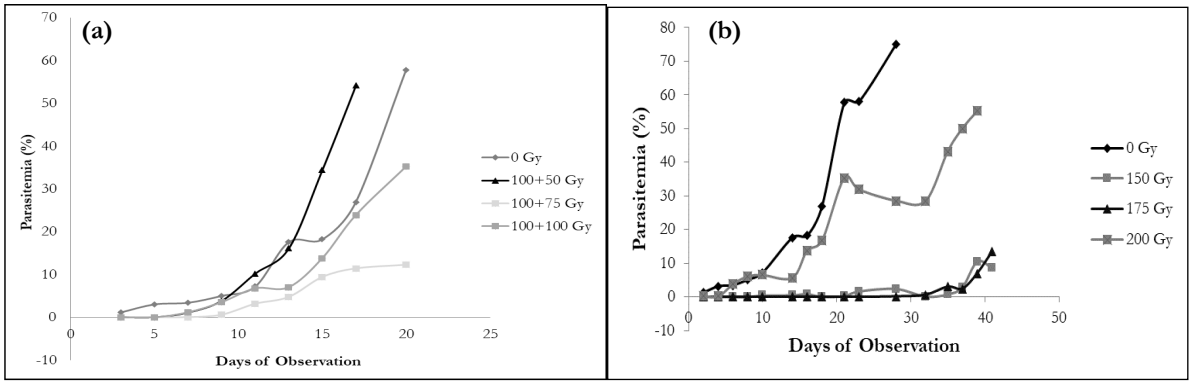

Figure 1. The fluctuation of parasitemia in mice blood on days post injection of *P. berghei* irradiated with (a) fractionated (100+50, 100+75 and 100+100 Gy) and its control (0 Gy), and (b) non-fractionated doses (single doses)

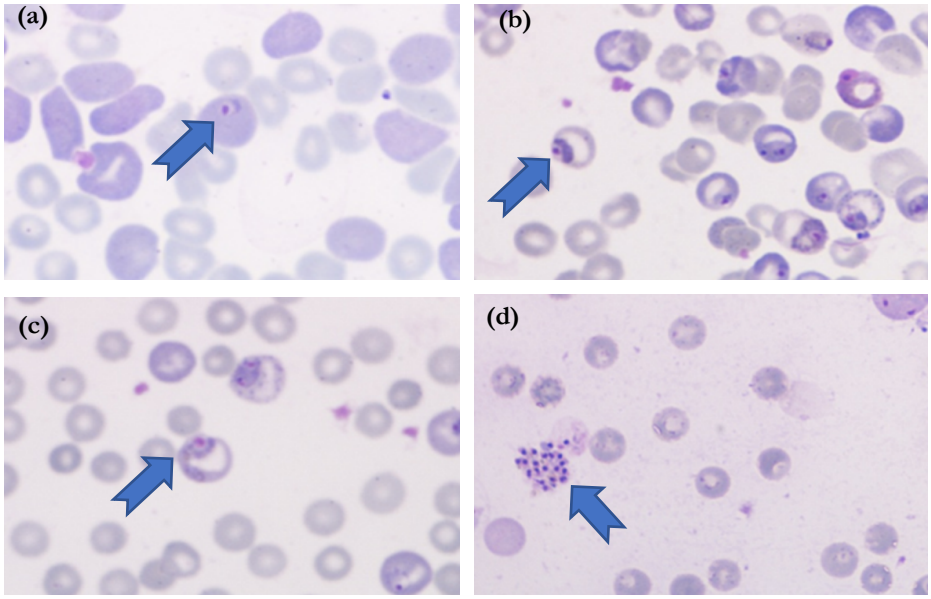

Figure 2. The microscopic views of parasitized red blood cells as a ring (a), young trophozoite (b), trophozoite (c), schizonts (d) forms in mouse body on day 9 post inoculated with 100+50 Gy irradiated *P. berghei*. Magnification of 1000 times for the original picture

DISCUSSION

Malaria parasites are found in every terrestrial habitat on all the warm continents of the world. Although research has contributed significantly in the understanding of immunity towards malaria, there are still considerable gaps in the knowledge (Tizifa *et al.*, 2018), mainly, similar with the tasks of BATAN, in the determination of optimal dose of radiation to attenuate parasites. The development

of malaria vaccines is still one of the primary goals of tropical diseases research. In this article, it is provided the first study on growth and examine the infectivity alterations in intraerythrocytic *P. berghei* treated with fractionated doses of γ-radiation to produce vaccine materials. Most tissues show a sparing effect of dose fractionation so that each dose for a given endpoint are lower if the dose is fractionated or divided into several parts rather than when given as a single dose (ICRP Publication, 2011; Stewart *et al.*, 2012). But this fractionated dose did not inhibit the growth of the parasite.

Malaria vaccination attempts with life, attenuated, killed or lysed parasites, as well as, different antigenic fractions of the parasite, have been conducted in many parts of the world with varying success (Hoffman *et al.*, 2010; Luke and Hoffman, 2003). The development of vaccines in the future will not only have to consider all life-cycle stages of the parasite that need to be targeted but will also have to take into account which immune responses need to be induced and in which tissue sites (Draper *et al.*, 2018; Elsaid *et al.*, 1999). Development of a successful vaccine against malaria has in part, been hampered by the complex life cycle of malaria parasite during which the microorganism displays many antigenic forms that elude stage-specific immune responses. The *in vitro* and *in vivo* experiments of parasite growth inhibitory activity is widely used as a surrogate marker for malaria vaccine efficacy (Hoffman *et al.*, 2010; Duncan *et al.*, 2011).

As seen in the results that a fractionation dose of irradiation did not suppress the parasite growth. In the previous study, a dose of gamma rays at 150 and 175 Gy given as single dose were effective in suppressing the parasitemia in mice (Darlina, 2011). It has been known that γ-ray causes many changes in the parasite which is ranged between decreasing its ability to cause infection and retaining its antigen effect to killing this parasite by using these high doses of irradiation and given at once (Cockburn *et al.*, 2010).

This experimental focused on the infectivity of parasites that were irradiated with doses that were fractionated. Fractionation is a method for curing cancer using radiation therapy. When the total dose of radiation is divided into several smaller doses and for several times, there are fewer toxic effects on healthy cells or fewer number of cells will die. This would maximize the effect of radiation on cancer and minimizes the negative side effects on normal cells. Experiments have found that if the absorbed dose of radiation increases then the number of survived cells will decrease. This is due to self-repair mechanisms which repair the damaged DNA and other macromolecules such as proteins. Thus when the total dose of radiation is divided into several smaller doses, there are fewer toxic effects on healthy cells (Alan and Ahmed, 2011; Moding *et al.*, 2013). That is why in this experiment the virulence of parasites was still high upon a lower dose of irradiation. This phenomenon is not applicable for attenuating parasites.

As mentioned above, basics of fractionation irradiation are that a dose is divided into several fractions (in this experiment only two fractions) that spares normal tissues where there is a repair of sublethal damage between dose fractions and repopulation of living cells. As in cancer therapy, however, dividing a radiation dose into several fractions would increase damage to the cancer cells, giving a chance to reoxygenation of tumor environment and induction of new combination of the genetic material of cells into radiosensitive phases of the cell cycle between dose fraction. Fractionation also means that there is a prolongation of treatment that reduces early reactions.

Cell radiobiology is a complex system where the response (radiosensitivity) of cell depends not only on the dose and dose rate of radiation but also on some other physical and biological factors such as linear energy transfer (LET), relative biological effectiveness (RBE), repair system, oxygenation, cell cycle, and dose fractionation. Other agents that given concurrently as modifier or radiosensitizer (*e.g.* chemoradiotherapy) is also another factor. It is important to be aware of basic

radiobiological models which can be used to describe the effects of radiation dose and fractionation. In this experiment, 5 minutes as the interval time of two fractions of irradiation was assumed not affecting the infectivity of parasites but rather the lower dose delivered 2 times that determined their infectivity (IAEA, 2014; Khorramizadeh *et al.*, 2017).

CONCLUSIONS

In summary, the experimental data showed that fractionated dose of radiation did not effectively attenuate the parasites where they were still growing in the blood of mouse, where 100+75 Gy is more effective compared to 100+50 and 100+100 Gy. Mice injected with irradiated parasites that were fractionated had a shorter survival time compared to the corresponding non-fractionated one.

REFERENCES

Alan, P., M. Ahmed. 2011. Hypofractionation: Scientific Concepts and Clinical Experiences. 1st. Ed. LimiText Publishing, Ellicot City.

Anonimous, Radiation Weakened Parasites: Possible New Malaria Vaccine? Retrieved on Saturday, November 10, 2007 at 1:20:29 PM (http://www.medindia.net/news/Radiation-Weakened-Parasites-Possible-New-Malaria-Vaccine-29169-1.htm.

Bijker, E.M., S. Borrmann, S.H. Kappe, B. Mordmuller, B.K. Sack, S.M. Khan. 2015. Novel approaches to whole sporozoite vaccination against malaria. Vaccine, 33(52):7462-7468.

Cabiscol, E., J. Tamarit, J. Ros. 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. International Microbiology, 3:3–8.

Carlton, J.M., S.V. Angiuoli, B.B. Suh, T.W. Kooij, M. Pertea, J.C. Silva M.D. Ermolaeva, J.E. Allen, J.D. Selengut, H.L. Koo, J.D. Peterson, M. Pop, D.S. Kosack, M.F. Shumway, S.L. Bidwell, S.J. Shallom, S.E. van Aken, S.B. Riedmuller, T.V. Feldblyum, J.K. Cho, J. Quackenbush, M. Sedegah, A. Shoaibi, L.M. Cummings, L. Florens, J.R. Yates, J.D. Raine, R.E. Sinden, M.A. Harris, D.A. Cunningham, P.R. Preiser, L.W. Bergman, A.B. Vaidya, L.H. van Lin, C.J. Janse, A.P. Waters, H.O. Smith, O.R. White, S.L. Salzberg, J.C. Venter, C.M. Fraser, S.L. Hoffman, M.J. Gardner, D.J. Carucci. 2002. Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. Nature, 419:512-519.

Cockburn, I.A., Y.C. Chen, M.G. Overstreet, J.R. Lees, N. van Rooijen, D.L. Farber, F. Zavala. 2010. Prolonged antigen presentation is required for optimal CD8+ T cell responses against malaria liver stage parasites. PLoS Pathogens, 6(5): e1000877.

Darlina. 2011. Rodent malaria parasite as model in vaccine research with nuclear technique. Alara Bulletin, 13(2): 53-60.

Draper, S.J., B.K. Sack, C.R. K ing, C.M. Nielsen, J.C. Rayner,M.K. Higgins, C.A. Long, R.A. Seder. 2018. Malaria Vaccines: Recent Advances and New Horizons. Cell Host and Microbe, 24(1):43–56.

Duncan, C.J.A., S.H. Sheehy, K.J. Ewer, A.D. Douglas, K.A. Collins, F.D. Halstead, S.C. Elias, P.J. Lillie, K. Rausch, J. Aebig, K. Miura, N.J. Edwards, I.D. Poulton, A. Hunt-Cooke, D.W. Porter, F.M. Thompson, R. Rowland, S.J. Draper, S.C. Gilbert, M.P. Fay, C.A. Long, D. Zhu, Y. Wu, L.B. Martin, C.F. Anderson, A.M. Lawrie, A.V. Hill, R.D. Ellis. 2011. Impact on malaria parasite multiplication rates in infected volunteers of the protein-in-adjuvant vaccine AMA1-C1/Alhydrogel+CPG 7909. PLoS ONE, 6(7):e22271.

Elsaid, M.M.A., R.W.A. Vitor, F.J.G. Frézard, M.S. Martins. 1999. Protection against toxoplasmosis in mice immunized with different antigens of *Toxoplasma gondii* incorporated into liposome, Brazilian Memorias Instituto Oswaldo Cruz, 94(4):485-490.

Feachem, R.G., A.A. Phillips, J. Hwang, C. Cotter, B. Wielgosz, B.M. Greenwood, O. Sabot, M.H. Rodriguez, R.R. Abeyasinghe, T.A. Ghebreyesus, R.W. Snow. 2010. Shrinking the malaria map: progress and prospects. Lancet, 376:1566-1578.

Fulda, S., A.M. Gorman, O. Hori, A. Samali. 2010. Cellular stress responses: cell survival and cell death. International Journal of Cell Biology, 2010: 214074.

Gerald, N.J., V. Majam, B. Mahajan, Y. Kozakai, S. Kumar. 2011. Protection from experimental cerebral malaria with a single dose of radiation attenuated, blood-stage *Plasmodium berghei* parasites. PLoS One., 6: e24398.

Hall, E.J., D.J. Brenner. 1991. The dose-rate effect revisited: radiobiological considerations of importance in radiotherapy. International Journal of Radiation Oncology Biology Physics, 21(6):1403-1414.

Han, W., K.N. Yu. 2009. Response of cells to ionizing radiation. Advances in Biomedical Sciences and Engineering. SC Tjong (Ed.). Bentham Sciences Publisher Ltd., Hongkong. pp. 204-262.

Hoffman, S.L., P.F. Billingsley, E. James, A. Richman, M. Loyevsky, T. Li, S. Chakravarty, A. Gunasekera, R. Chattopadhyay, M. Li, R. Stafford, A. Ahumada, J.E. Epstein, M. Sedegah, S. Reyes, T.L. Richie, K.E. Lyke, R. Edelman, M.B. Laurens, C.V. Plowe, B.K.L. Sim. 2010. Development of a metabolically active, non-replicating sporozoite vaccine to prevent *Plasmodium falciparum* malaria. Human Vaccines, 6:97-106.

ICRP Publication. 2011. Early and late effects of radiation in normal tissues and organs: threshold doses for tissue reactions and other non-cancer effects of radiation in a radiation protection context, The International Commission on Radiological Protection. Ottawa. pp.17-20.

International Atomic Energy Agency. 2014. Radiation Protection in Radiotherapy: Part 3 Biological Effects, IAEA Training Material on Radiation Protection in Radiotherapy. Vienna Austria.

Khorramizadeh, M., A. Saberi, M. Tahmasebi–birgani, P. Shokrani, A. Amouhedari. 2017. Impact of prolonged fraction delivery time modelling stereotactic body radiation therapy with high dose hypofractionation on the killing of cultured ACHN renal cell carcinoma cell line. Journal of Biomedical and Physical Engineering, 7(3): 205–216.

Luke, T.C., S.L. Hoffman. 2003. Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine. Journal of Experimental Biology, 206:3803-3808.

Mahmoudi, S., H. Keshavarz. 2018. Malaria vaccine development: the need for novel approaches: A review article. Iranian Journal of Parasitology, 13(1):1–10.

Mettler, F.A. Jr., G.L. Voelz. 2002. Major radiation exposure – what to expect and how to respond. New England Journal of Medicine, 346:1554-1561.

Moding, E.J., M.B. Kastan, D.G. Kirsch. 2013. Strategies for optimizing the response of cancer and normal tissues to radiation. Nature Review Drug Discovery, 12(7): 526–542.

Oakley, M.S., N. Gerald, V. Anantharaman, Y. Gao, V. Majam, B. Mahajan, P.T. Pham, L.L. Cole, T.G. Myers, T.F. McCutchan, S.L. Morris, L. Aravind, S. Kumar. 2013. Radiation-induced cellular and molecular alterations in asexual intraerythrocytic *Plasmodium falciparum*. Journal of Infectious Diseases, 207(1):164–174.

Ouattara, A., M.B. Laurens. 2015. Vaccines against malaria. Clinical Infectious Diseases, 60(6): 930–936.

Ramani, S., S.C. Parija, J. Mandal, A. Hamide, V. Bhat. 2016. Detection of chloroquine and artemisinin resistance molecular markers in *Plasmodium falciparum*: A hospital based study. Tropical Parasitology, 6(1):69–77.

Seo, H.S. 2015. Application of radiation technology in vaccines development. Clinical and Experimental Vaccine Research, 4(2):145–158.

Stewart, F.A., A.V. Akleyev, M. Hauer-Jensen, J.H. Hendry, N.J. Kleiman, T.J.Macvittie, B.M. Aleman, A.B. Edgar, K. Mabuchi, C.R. Muirhead, R.E. Shore, W.H. Wallace. 2012. ICRP publication 118: ICRP statement on tissue reactions and early and late effects of radiation in normal tissues and organs--threshold doses for tissue reactions in a radiation protection context. Annals of the ICRP., 41(1-2):1-322.

Syaifudin, M., D. Tetriana, Darlina, S. Nurhayati. 2011. The feasibility of gamma irradiation for developing malaria vaccine. Atom Indonesia, 37(3):91-101.

Syaifudin, M., Darlina, T. Rahardjo, D. Tetriana, S. Nurhayati, H.E.S. Surniyantoro, T. Kisnanto. 2013. Efectiveness of gamma rays in attenuating rodent malaria parasites of *Plasmodium berghei* in blood of mice. Atom Indonesia, 39(1): 19-23.

Tizifa, T.A., A. N. Kabaghe, R.S. McCann, H. van den Berg, M.V. Vugt, K.S. Phiri. 2018. Prevention efforts for malaria. Current Tropical Medicine Reports, 5(1):41–50.

World Health Organization. 2018. World Malaria Report 2018. Geneva.